

# Nano Research

## Electrophysiological Assessment of Primary Cortical Neurons Genetically Engineered using Iron Oxide Nanoparticles --Manuscript Draft--

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<b>Abstract:</b>	<p>The development of safe technologies to genetically modify neurons is of high interest in regenerative neurology, for both translational and basic science applications. Such approaches have conventionally been heavily reliant on viral transduction methods which have safety and production limitations. Magnetofection (magnet-assisted gene transfer using iron oxide nanoparticles as vectors) has emerged as a highly promising non-viral alternative for safe and reproducible genetic modification of neurons. Despite the high promise of this technology, there is an important gap in our knowledge of the safety of this approach, namely, whether it alters neuronal function in adverse ways such as by altering neuronal excitability and signalling. We have investigated the effects of magnetofection in primary cortical neurons by examining neuronal excitability using the whole cell patch clamp technique. We found no evidence that magnetofection alters the voltage-dependent sodium and potassium ionic currents that underpin excitability. Our study provides important new data supporting the concept that magnetofection is a safe technology for bioengineering of neuronal cell populations.</p>	
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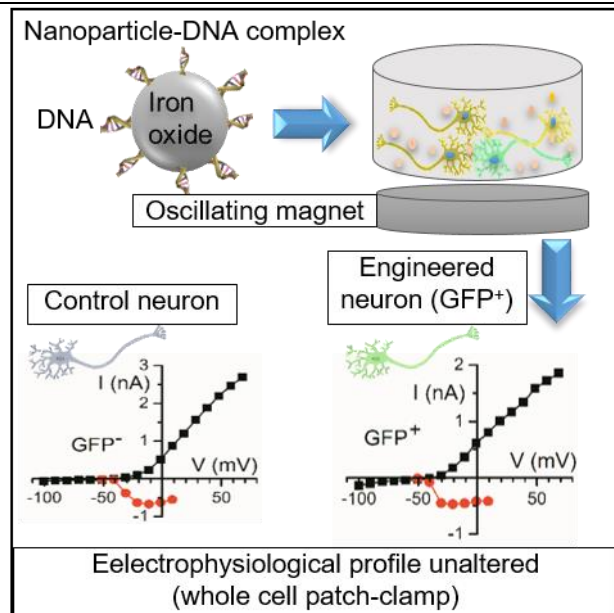
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An electrophysiological study of neurons genetically engineered using iron oxide nanoparticles reveals the high safety of the approach.

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# Electrophysiological Assessment of Primary Cortical Neurons Genetically Engineered using Iron Oxide Nanoparticles

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## ABSTRACT

The development of safe technologies to genetically modify neurons is of high interest in regenerative neurology, for both translational and basic science applications. Such approaches have conventionally been heavily reliant on viral transduction methods which have safety and production limitations. Magnetofection (magnet-assisted gene transfer using iron oxide nanoparticles as vectors) has emerged as a highly promising non-viral alternative for safe and reproducible genetic modification of neurons. Despite the high promise of this technology, there is an important gap in our knowledge of the safety of this approach, namely, whether it alters neuronal function in adverse ways such as by altering neuronal excitability and signalling. We have investigated the effects of magnetofection in primary cortical neurons by examining neuronal excitability using the whole cell patch clamp technique. We found no evidence that magnetofection alters the voltage-dependent sodium and potassium ionic currents that underpin excitability. Our study provides important new data supporting the concept that magnetofection is a safe technology for bioengineering of neuronal cell populations.

## KEYWORDS

Transfection, magnetic nanoparticle, GFP, whole-cell patch clamp, fluorescence microscopy

## 1. Introduction

The endogenous repair capacity of the central nervous system (CNS) following injury or disease is limited; the low capacity of neurons to regenerate is a

major contributor to this phenomenon [1]. Accordingly, extensive basic scientific research has been directed towards gaining an enhanced understanding of neuronal biology, including the

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roles of key genes and proteins that can improve neuronal survival and function, in order to inform the development of novel therapeutic interventions [2]. A major technical issue to consider in this context, is the ability to safely and successfully deliver genetic material to target neuronal populations. Post-mitotic neurons are challenging in this regard, being both hard to transfect and showing high sensitivity to alterations in their microenvironment, necessitating minimal cell manipulation during the genetic modification procedures [3]. To this end, both viral and non-viral approaches have been used for targeted neuronal gene delivery [4]. The key factors that determine the suitability of a methodology for genetic engineering include (i) transfection efficiency achieved, (ii) reproducibility for a given cell type, (iii) cost, (iv) technical complexity, (v) production scalability and (vi) cytotoxicity. In this regard, non-viral gene delivery systems such as electroporation, lipofection, and nucleofection can be associated with membrane damage and high cell loss, considerable expense and low transfection [4,5]. Viral methods such as adenoviruses, lentiviruses and herpes simplex virus can achieve high transfection levels but can be associated with insertional mutagenesis, contaminant related toxicity, and expensive and time consuming production procedures [5].

A promising non-viral alternative for neuronal gene transfer that has emerged in recent years, involves the use of iron oxide magnetic nanoparticles (MNPs) with chemically adaptable surfaces, allowing bound genetic material to be targeted to cells by application of static or oscillating magnetic fields (Magnetofection) [6]. This approach is rapid, technically simple, safe and low cost. It has been used extensively for genetic modification of both neuronal cell lines and primary neurons, including for delivery of physiologically relevant biomolecules. For example, reporter genes (such as GFP) have been delivered in transfection studies of neurons derived from stem cells [7], as well as in embryonic primary motor neurons [8], in order to test the suitability of this method at different developmental stages. The approach has also been used to study the localization

and axonal transport of the spinal muscular atrophy protein (SMN) in motor neurons [8]. Other studies have successfully utilised magnetofection to overexpress the gene encoding scaffolding protein RanBP9 in primary cortical neurons, with a resultant reduction in dendritic spine density [9]. Further, this technique has been applied in gene knockdown studies to decrease the synaptic GTPase activating protein (SynGAP) expression via the sustained expression of short hairpin RNA (shRNA) in neurons for up to six days [10].

This bioengineering strategy has now been successfully used for a wide range of neuronal transfection applications by laboratories worldwide. Despite its high utility, it should be noted that investigations into the potential adverse effects of this technology on neuron biology rely almost exclusively on relatively simple histological readouts such as cell adherence, phenotypic evaluation, live/dead staining, viable cell counts using flow cytometry, mitochondrial toxicity assays and lactate dehydrogenase (LDH) activity [11]. However, a crucial knowledge gap currently exists in relation to magnetofection technology for neuronal applications, namely, *whether this bioengineering approach alters neuronal excitability*. This is a critical issue to address as nanoparticles such as carbon black (CB), hematite ( $\text{Fe}_2\text{O}_3$ ), and titanium dioxide ( $\text{TiO}_2$ ) were found to alter spiking patterns in primary murine cortical neurons cultured on microelectrode array neuro-chips in a concentration dependent manner [12]. The acute electrophysiological effects were studied after particle addition to cells. Multi-parametric assessment of changes in electrical activity showed a decrease in the number of action potentials, with altered patterns in terms of spike and burst rate. Silver nanoparticles were also found to reduce sodium current size and action potential amplitude in a concentration-dependent manner in whole-cell recordings from hippocampal CA1 neurons in brain slices [13]. A 30% decrease in amplitude and -6mV shift in the activation curve of sodium current was observed.

To address this important issue we have conducted for the first time an electrophysiological analysis

(using single cell patch clamp recordings) into the effect of magnetofection on the voltage-dependent ion channels that underpin excitability in primary cortical neurons.

## 2. Materials and methods

The care and use of animals was in accordance with the Animals (Scientific Procedures) Act of 1986 (United Kingdom) with approval by the local ethics committee.

### 2.1 Equipment and reagents

Cell culture-grade plastics, Hanks Balanced salt solution (HBSS; calcium and magnesium free), B27 serum-free supplement [14], glutaMAX-I, penicillin and streptomycin (P/S) and TrypLE (synthetic trypsin, with phenol) were obtained from Fisher (Loughborough, UK). Chemicals for electrophysiology, phosphate buffer saline (PBS) and N-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid (HEPES) were from Sigma-Aldrich (Poole, Dorset, UK). Neurobasal, Dulbecco's Modified Eagle's medium (DMEM) and F12 media were from Invitrogen (Paisley, Scotland, UK). Deoxyribonuclease I (DNase I) was from Roche (Welwyn, UK). Antibodies: rabbit anti-glial fibrillary acidic protein (GFAP; Z0334; Dako Cytomation, Ely, UK), rabbit anti- $\beta$ -tubulin (Tuj-1; MRB-435P; Covance, Princeton, USA). Cy3- and fluorescein isothiocyanate (FITC)-conjugated secondary antibodies were from Jackson ImmunoResearch Laboratories Ltd (West grove, PA, USA). Vectashield mounting medium with 4, 6-diamidino-2-phenylindole (DAPI, nuclear marker) was from Vector Laboratories (Peterborough, UK). Fetal bovine serum (FBS) and normal donkey serum were from Dutscher Scientific (UK) and Stratech Scientific (UK) respectively. The magnefect-nano system, comprising an array of NdFeB magnets (grade N42; field strength of  $421 \pm 20$  mT) matching the wells of culture plates, was from nanoTherics Ltd (Stoke-on-Trent, UK [15]). This array oscillates horizontally beneath the culture plate, with programmable frequency and oscillation. Neuromag MNPs were from Oz Biosciences

(Marseilles, France). pMAXGFP plasmid [size 3.5 kb; encodes green fluorescent protein (GFP)] was from Amara Biosciences (Cologne, Germany). Tetrodotoxin (TTX) was from Tocris (UK), borosilicate glass for patch electrodes from Harvard Instruments, blue excitation LED from Cairn Instruments, Optimos camera from Q Imaging, patch clamp amplifier from HEKA and the Signal software and computer interface (Power 1401) was from CED.

### 2.2 Culture of primary cortical neurons

Neuronal cells were derived from embryonic day 18 (E18) CD1 mouse cortex. Pregnant mice were killed by cervical dislocation and the uterus was exposed by cutting the abdominal cavity using scissors sterilized with 70% ethanol. The uterus was placed in a petri dish containing ice-cold dissection medium (97.5% HBSS, 15 mM HEPES, 50 U mL<sup>-1</sup> penicillin, 50  $\mu$ g mL<sup>-1</sup> streptomycin). The embryos were decapitated very quickly into ice-cold HBSS. Cortical tissue was extracted and minced using a sterile scalpel blade and incubated with 250  $\mu$ L of TrypLE and incubated for 20 min with shaking at 37°C. The digestion was stopped by adding 1 ml of FBS and cells were dissociated mechanically for 1-2 min and then strained (70  $\mu$ m followed by 40  $\mu$ m). Cells were seeded at  $1 \times 10^5$  cells mL<sup>-1</sup> in 24 well plates with 400  $\mu$ L growth medium (96% Neurobasal medium, 2% B-27, 2 mM glutaMAX-I, 50 U mL<sup>-1</sup> penicillin, 50  $\mu$ g mL<sup>-1</sup> streptomycin) placed in standard humidified incubator (37°C, 95% air/5% CO<sub>2</sub>), and the cells allowed to adhere to the coverslip (<1 h). Then the medium was replaced with 600  $\mu$ L fresh growth medium, with a 50% medium change every 2-3 days.

### 2.3 Transfection (magnetofection)

At 7 days in vitro (DIV), medium was replaced with 225  $\mu$ L antibiotic-free growth medium. MNP-plasmid complexes were formed by diluting (per well) 178 ng pMAXGFP plasmid in 75  $\mu$ L 1:1 DMEM: F12 and adding 0.62  $\mu$ L Neuromag (20 min, room temperature). This mixture was added to the cells dropwise with gentle swirling of the plate. 75

1  $\mu$ L DMEM: F12 medium free of complex were added  
2 to the controls. Plates were then incubated for 30 min  
3 on the magnefect-nano array system [24 magnets,  
4 matching the culture plate; frequency (F) = 4 Hz;  
5 amplitude = 200 nm], then incubated for 24-72 h in  
6 the absence of the magnetic field [7].  
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## 10 **2.4 Viability (live/dead staining)**

11 Viability was assessed at 48 h post-magnetofection.  
12 Cultures were washed gently with PBS, then exposed  
13 to 4 mM calcein-AM (fluorescent green; indicates live  
14 cells) and 2 mM ethidium homodimer-1 (fluorescent  
15 red; indicates dead cells) for 5 min (37°C), washed  
16 twice with PBS, then imaged immediately.  
17 Magnetofection-induced toxicity was assessed by  
18 determining the percentage of viable (calcein positive)  
19 cells.  
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## 25 **2.5 Immunocytochemistry**

26 Samples were fixed 48 h post-magnetofection (4%  
27 paraformaldehyde in PBS; 20 min; room temperature)  
28 before incubation with blocking solution (5% normal  
29 donkey serum, 0.3% Triton-X in PBS; 30 min), then  
30 primary antibodies in blocking solution (Tuj1 1:1000;  
31 anti-GFAP 1:500; 4°C; overnight). Samples were then  
32 washed three times with PBS, blocked (30 min) and  
33 incubated with secondary antibodies in blocking  
34 solution (1:200). Samples were washed three times in  
35 PBS, then mounted with the nuclear stain DAPI.  
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## 41 **2.6 Whole-cell electrophysiological recording**

42 Whole-cell recordings were made at room  
43 temperature, using patch pipette electrodes pulled  
44 with shanks coated with wax to reduce pipette  
45 capacitance. Neurons cultured on circular coverslips  
46 were secured, using a small drop of Sylgard, in the  
47 center of the lid of a 35 mm cell culture dish that  
48 acted as a chamber. The chamber was filled with  
49 Neurobasal medium and placed on the stage of an  
50 Olympus BX51 microscope fitted with an  $\times 40$   
51 water-immersion objective (Olympus, NA = 0.8).  
52 Neurons were identified as GFP positive / negative  
53 by the presence / absence of GFP fluorescence under  
54 appropriate epi-fluorescence illumination. Images  
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were taken with a Watec 902B camera or Optimos  
camera mounted on the microscope. Voltage-clamp  
protocols were run using Signal software with a  
Power 1401 interface, a patch clamp amplifier (EPC7)  
and a standard laboratory computer. The patch  
pipette filling solution contained (mM): KCl 140,  
Na<sub>2</sub>ATP 2.5, MgCl<sub>2</sub> 3.5, EGTA 1, and HEPES 10,  
buffered to pH 7.4 with KOH. The large end of the  
pipette was gently fire polished before filling and  
attachment to the head stage. Pipettes had a  
resistance of 4 M $\Omega$  when filled. TTX was prepared as  
a 1 mM stock solution in citrate buffer, frozen in  
aliquots, and dissolved in Neurobasal medium on  
the day of use. It was applied to the neuron from a  
second pipette positioned about 10  $\mu$ m from the cell  
body and ejected using a picopump (PV820, WPI)  
either manually or via a trigger pulse.

## **2.7 Fluorescence microscopy and image analysis**

Fluorescence micrographs were taken using an Axio  
Scope A1 (equipped with an Axio Cam ICc1 digital  
camera and AxioVision software, release 4.7.1; Carl  
Zeiss Micro Imaging GmbH, Goettingen, Germany),  
then merged using Photoshop (Adobe, USA). To  
evaluate transfection levels, each DAPI-positive  
nucleus was identified as neuronal (Tuj1<sup>+</sup>), astrocytic  
(GFAP<sup>+</sup>), or undetermined (Tuj1<sup>-</sup>/GFAP<sup>-</sup>), and scored  
as 'transfected' if GFP<sup>+</sup>. Pyknotic nuclei (indicative of  
necrotic or apoptotic cells) were identified by  
chromosomal condensation, and reported as a  
percentage of all (healthy plus pyknotic) nuclei.

## **2.8 Data analysis**

For the toxicity assays (live/dead and percentage  
pyknotic nuclei), three sets of micrographs (two from  
the edges and one from the centre of each coverslip;  
each set comprised of counterpart red, green, blue  
and phase contrast images) were captured and  
merged. Values are presented as mean  $\pm$  standard  
error of the mean. For culture data, *n* indicates the  
number of cultures (each obtained from different  
pregnant females); for electrophysiological data, *n*  
indicates the number of cells recorded (derived from  
eight separate litters). Data were analyzed by

two-tailed *t*-tests ( $p < 0.05$  considered significant) using either Prism (version 6.0; GraphPad, USA) or Origin 2016 (Origin Labs). Electrophysiological recordings were analysed using Signal and Origin software. Leak subtraction and capacitive transient reduction was performed where indicated by the addition of currents recorded in response to positive (depolarizing) voltage clamp steps to currents recorded in response to small (10-40 mV) negative voltage clamp steps, scaled and averaged if necessary.

### 3. Results

#### 3.1 Culture characterization

The proportion of neurons (Tuj1<sup>+</sup>) and astrocytes (GFAP<sup>+</sup>) was consistent across cultures ( $25.6 \pm 3.2\%$  Tuj1<sup>+</sup>,  $58.0 \pm 4.5\%$  GFAP<sup>+</sup>;  $n = 3$ ) with  $16.3 \pm 7.7\%$  of nuclei being undetermined (Tuj1<sup>-</sup>/GFAP<sup>-</sup>). Neurons showed well-defined spherical somata with long processes that formed a complex network with adjacent neurons. Astrocytes were morphologically distinguished as (flat, membranous, unbranched or star-shaped; Figure 1).

#### 3.2 Transfection (magnetofection) efficiency

After magnetofection,  $3.2 \pm 1.2\%$  of viable cells were transfected neurons (GFP<sup>+</sup>/Tuj1<sup>+</sup>), and a further  $6.3 \pm 3.3\%$  expressed GFP but not Tuj1. These latter cells exhibited astrocytic morphologies (Figure 1a and d). Statistical analysis demonstrated no significant differences between these values.

#### 3.3 Safety assessments of particle toxicity in cortical neuronal culture

No differences were noted between neurons in magnetofected and control cultures with respect to morphology, percentage of live cells ( $59.3 \pm 9.2\%$ ,  $53.0 \pm 2.0\%$  respectively), number of neurons per micrograph ( $36.3 \pm 16.5$ ,  $35.6 \pm 10.7$ ), or percentage of pyknotic nuclei ( $38.6 \pm 3.0\%$ ,  $35.3 \pm 7.8\%$ ; Figure 1).

#### 3.4 Electrophysiological characterization

Neurons for whole cell recording were identified under normal light conditions by a bulb-shaped soma with long processes often forming a complex network, and under blue light (excitation wavelength 495 nm), by the fluorescence associated with GFP protein expression (Figure 2).

In voltage-clamp experiments, depolarising voltage-clamp steps produced biphasic currents comprising an early inward current, reaching a peak at around 2 ms after the step, followed by a late outward current that activated over 5-10 ms (Figure 2a and b). The early inward current was found to be rapidly blocked by TTX ( $25 \mu\text{M}$ ) suggesting that it was a voltage-dependent Na<sup>+</sup> current (Figure 2d). The late outward current, which was maintained for the duration of the voltage step, was likely to be carried by K<sup>+</sup> since its reversal potential was negative to -60 mV, based on outward tail current polarity at -60 mV (Figure 2a). Currents carried by K<sup>+</sup> would be expected have a reversal potential of -83 mV (the calculated K<sup>+</sup> equilibrium potential under our recording conditions). From a holding potential of -60 mV or -70 mV, the Na<sup>+</sup> and K<sup>+</sup> currents were first seen, in response to depolarising steps, at around -40 mV. The Na<sup>+</sup> current activated rapidly (1-3 ms) and was maximal at about -20 mV (Figure 2c). A comparison between GFP<sup>+</sup> and GFP<sup>-</sup> neurons revealed no differences between current amplitudes or membrane potential (Student's *t* test; Table 1).

In current clamp, most neurons exhibited a small spike following a step depolarising current injection (Figure 3a and b). The spikes were 15-30 mV in amplitude and did not reach or exceed 0 mV. We also noticed in a few cells spontaneous depolarisations that increased in amplitude with hyperpolarisation (Figure 3a and b). These presumably reflect excitatory synaptic activity.

### 4. Discussion

To our knowledge, this is the first study investigating the electrophysiological properties of magnetofected primary neurons. We find no evidence for any functional influence of magnetofection in these



1 relatively immature cortical neurons, based on our  
2 experiments demonstrating similar amplitudes and  
3 kinetics of the voltage-dependent  $\text{Na}^+$  and  $\text{K}^+$   
4 currents. These findings are corroborated by our  
5 histological observations that did not reveal adverse  
6 effects of the genetic modification protocols on key  
7 parameters of cell health. Our use of primary  
8 neurons has the advantage of eliminating possible  
9 confounding issues associated with cell lines such as  
10 their relative homogeneity and clonal nature and  
11 resistance to nano-toxicity along with genetic drift  
12 [16-18], enabling a more physiologically relevant  
13 study to be undertaken.

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19 Our observation that magnetofection did not affect  
20 the  $\text{Na}^+$  and  $\text{K}^+$  channels in cortical neurons is clearly  
21 an encouraging result in terms of its potential use in  
22 clinical applications employing cell or tissue  
23 transplants. It also provides an encouraging platform  
24 for basic science applications, where the effects of  
25 transgenes on neuronal function could be  
26 investigated in the absence of confounding issues,  
27 such as the decreased cell viability associated with  
28 some transfection techniques. Clearly, normal ion  
29 channel function is a prerequisite for normal  
30 neuronal function in any environment, including  
31 transplantation into a host. Even in the absence of  
32 functional synaptic contacts, a transplanted neuron's  
33 membrane potential, and ability to spike (all  
34 functions of ion channels), would be important for  
35 the health of the neuron and its ability to secrete  
36 chemicals or neurotransmitters. Our observation of  
37 apparently spontaneous excitatory synaptic  
38 potentials is significant since it implies that synaptic  
39 contacts are functional in these neurons. These  
40 factors are suggestive of a healthy and maturing cell  
41 culture amenable to gene manipulation by  
42 magnetofection. Future studies should assess more  
43 completely the signalling and synaptic properties of  
44 such bioengineered neurons. This would require  
45 both single neuron recordings and recordings of  
46 neuronal populations in order to build up a picture  
47 of function and signalling at the single cell and  
48 multi-cell levels. Given that ion channel dysfunction  
49 or channelopathy can lead to epileptic seizures and a

myriad of other problems [19], such information is  
vital before any type of clinical intervention using  
magnetofected neurons or glia can be successfully  
undertaken.

However, cellular uptake of metal or carbon-based  
nanoparticles has been associated with changes in  
spiking frequency and bursting patterns, based on  
neurochip extracellular recordings [12], and with  
alterations in  $\text{Na}^+$  current amplitude and activation  
range in whole-cell patch recordings [13]. These  
effects are clearly concentration-dependent and are  
subtle at low concentrations of nanoparticles,  
including iron oxide core MNPs [12]. It is possible  
that the conductive properties of the nanoparticle  
themselves may have an influence on cell excitability  
when using conductive materials such as carbon  
black or silver; future research will need to  
investigate this issue in further detail for the  
identification of safe nanomaterials. Comparison  
between studies in terms of metal or particle  
concentration is difficult in as much as uptake into  
the cells is highly dependent on particle size and cell  
type. The MNPs employed here, at ~160 nm diameter  
[20], and with an additional chemical envelope for  
plasmid attachment, are relatively large, but these  
and similar particles have a well-documented safety  
profile across a range of neural cell types [7, 15-18,  
20]. Further investigation should be directed towards  
more mature cells and to track changes  
post-transfection over longer time periods.

Transfection-based bioengineering strategies for  
neural repair will require both efficient and safe  
protocols. Our electrophysiological analysis of  
magnetofected cells was restricted to neurons clearly  
identified as expressing the reporter protein, hence  
the transfection levels obtained do not impact on the  
findings of our study. The overall culture transfection  
efficiency reported here (ca. 10%) is lower than that  
reported for various neuronal types including  
primary cortical neuronal cultures (up to 46%, using  
non-viral approaches combined with high DNA  
concentrations [8]). It should also be noted that the  
protocol employed here was safe over the time

course of our experiments in terms of cell survival and an apparent absence of pathological changes. Multiple factors can be predicted to influence transfection efficiency, including: developmental stage, conditions of cell culture (e.g. type of media used, physicochemical structure of vectors, and vector:DNA ratio) [8-10, 21]. These factors are likely to account for the differences in observations between studies.

It is well established that astrocytes show high levels of endocytotic activity in line with their homeostatic functions in the nervous system, resulting in documented 'competitive uptake dynamics' for nanoparticle uptake which in co-cultures would limit neuronal transfection [15, 16, 22]. Because of this, we chose cell culture conditions that would limit astrocyte numbers and proliferation. First, by deriving cultures from embryonic tissue, astrocyte numbers can be kept low, as it is estimated that >90% of cells at this developmental time point are neurons [23]. Second, the use of serum free medium can (i) enhance the purity of neuronal cultures by reducing astrocyte proliferation and (ii) provide greater definition of experimental conditions by removing confounding variation in serum composition [24-26] and eliminating the need to use chemical inhibitors of astrocytes such as arabinosylcytosine C (AraC) [27, 28]. Nevertheless, in our hands it proved difficult to eliminate the issue of astrocyte contamination completely; approximately 35% of the astrocyte population can re-enter the cell cycle after serum deprivation, likely accounting for the high proportions of astrocytes in these cultures [23].

In summary, we provide the first complementary electrophysiological and histological analyses supporting the concept that iron oxide nanoparticles and applied magnetic fields can be safely deployed for genetic modification of primary neurons for basic research and translational applications.

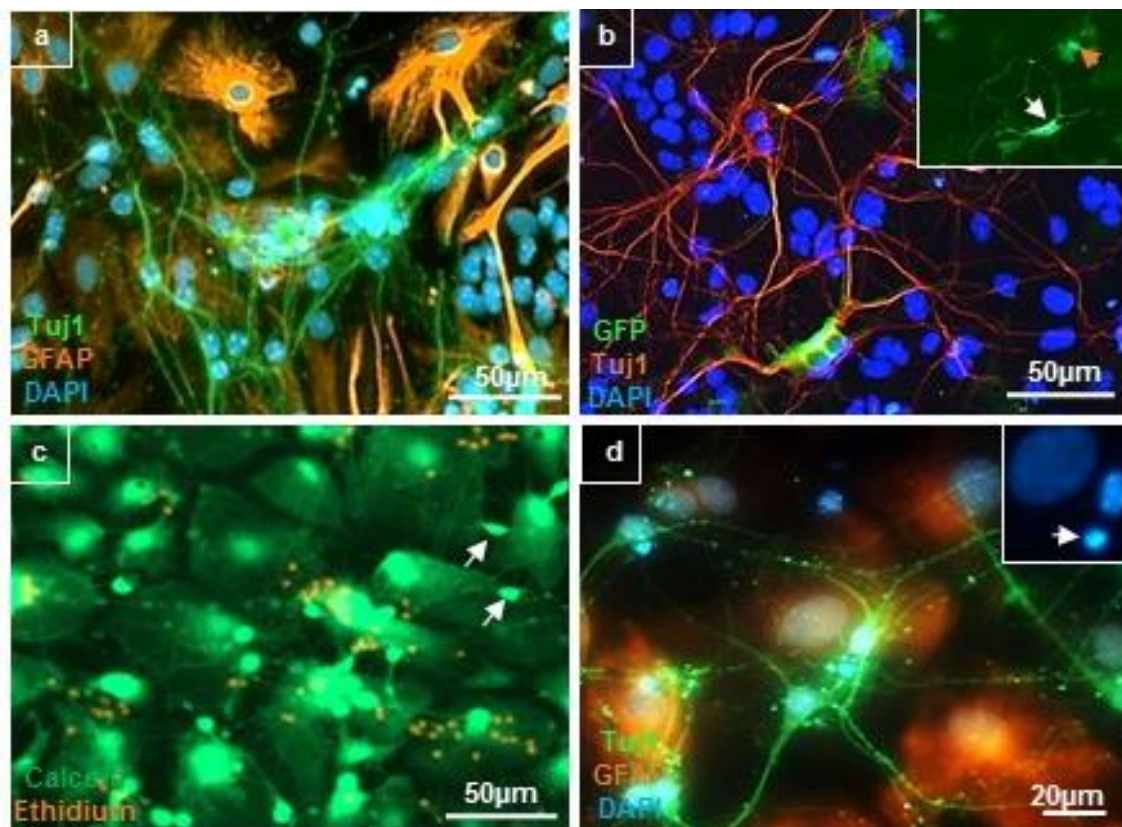
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**Figure 1. Magnetofection is safe for transfecting primary cortical neuronal cultures.** Fluorescence micrographs of cortical cultures after 7 days *in vitro*. (a) Neurons (Tuj1<sup>+</sup>) display small rounded soma with long processes, astrocytes (GFAP<sup>+</sup>) are flat, membranous and unbranched. (b) Neurons after magnetofection co-express Tuj1 and GFP. The inset shows a GFP<sup>+</sup> astrocyte (orange arrow) and a Tuj1<sup>+</sup>/GFP<sup>+</sup> neuron (white arrow). (c) Cell viability assay showing viable cells in culture (green) and dead cells (orange). (d) Pyknotic nuclei show condensed chromatin (white arrow, inset) in cortical co-culture. The inset shows the same arrowed neuron showing the condensed chromatin more clearly.

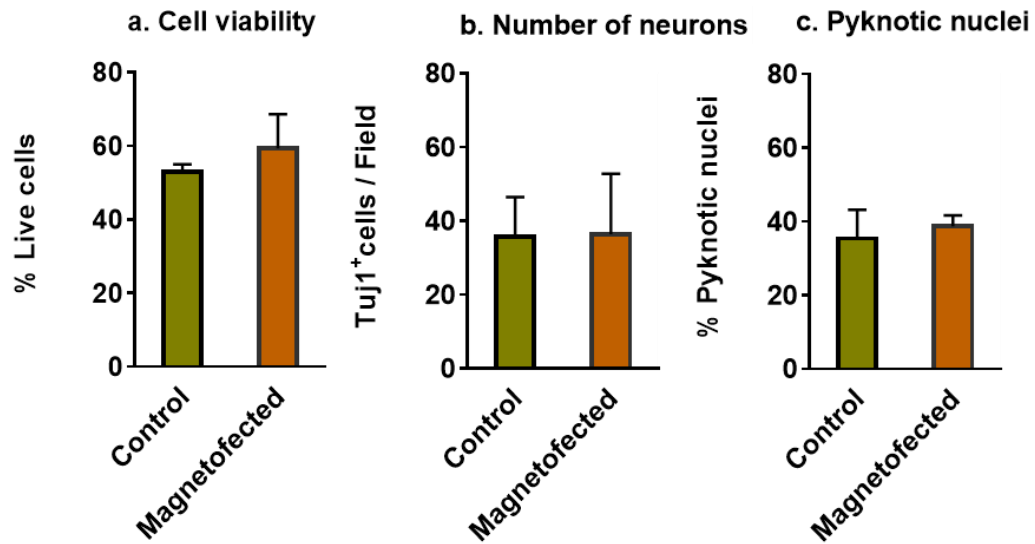
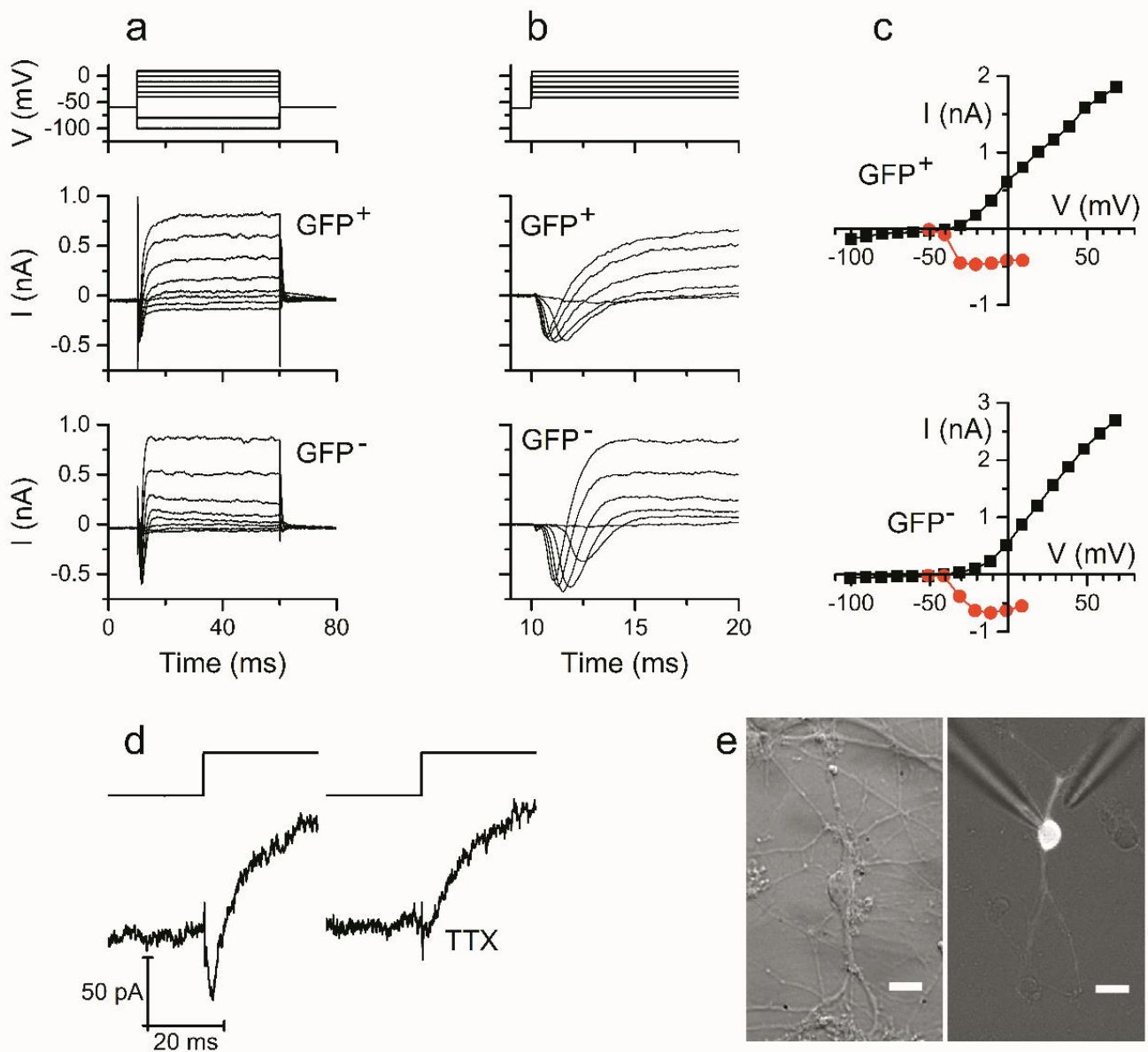


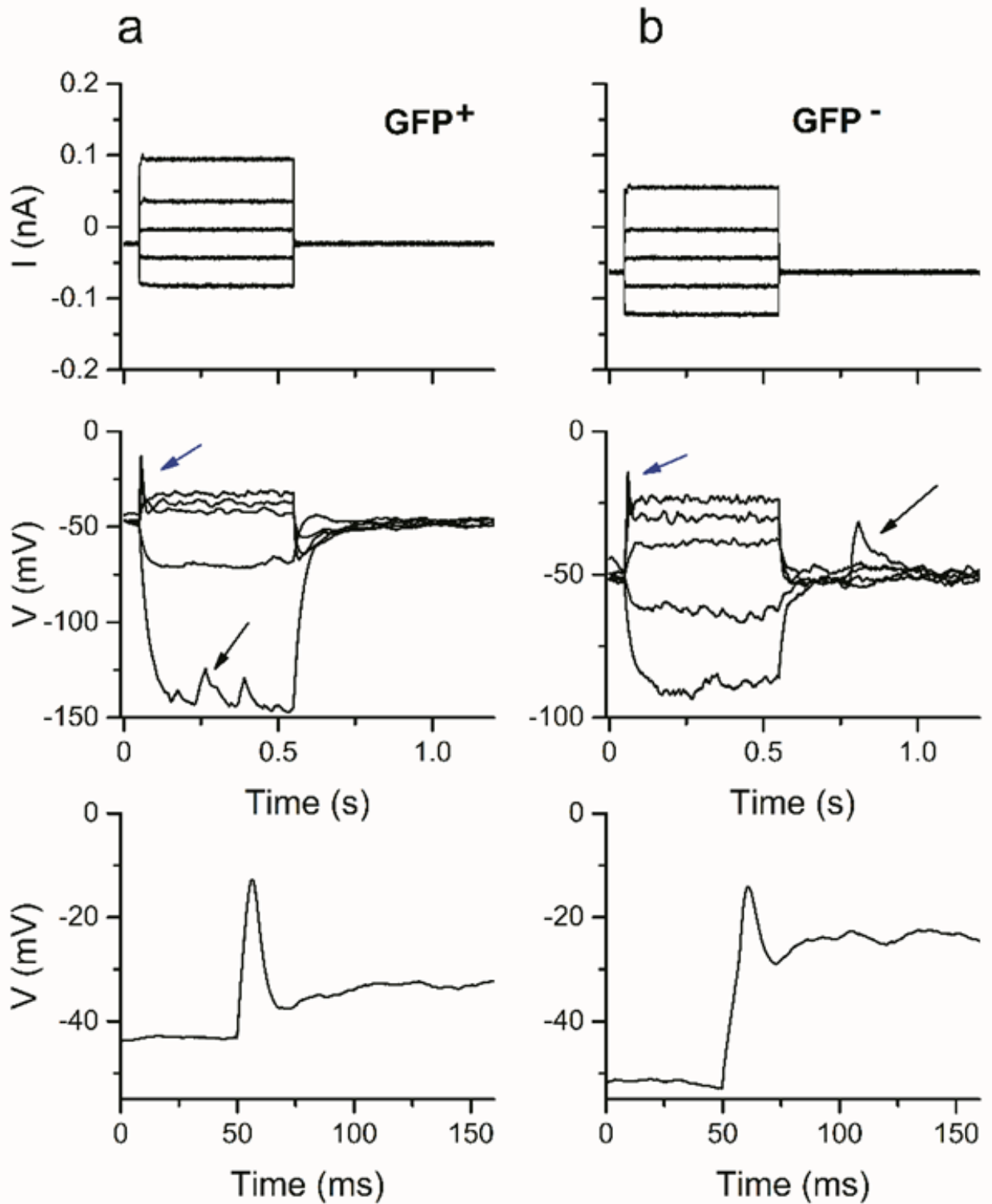
Figure 2. **Graphs depicting the safety of magnetofection technique.** No alterations are seen in (a) cell viability. (b) neuronal number. (c) Percentage of pyknotic nuclei (n = 3).



**Figure 3. Representative ionic currents seen in response to voltage steps from a holding potential of -60 mV from both magnetofected (GFP<sup>+</sup>) and non-magnetofected (GFP<sup>-</sup>) neurons.** (a) Voltage steps (top) and corresponding ionic currents are shown for GFP<sup>+</sup> and GFP<sup>-</sup> neurons as indicated (bottom two rows). (b) Leak-subtracted records shown on a faster time scale to reveal early inward (Na<sup>+</sup>) currents followed by late outward (K<sup>+</sup>) currents. Holding currents have been subtracted. Same cells as (a). In (a) and (b) the horizontal (time) and vertical scales are shown at the bottom and on the left respectively. (c) I-V plots of early inward and late outward currents from GFP<sup>+</sup> (top) and GFP<sup>-</sup> neurons (bottom). The late currents (squares) were measured from the records shown in (a) at the 40ms time point, the early currents (circles) were measured as peak inward currents from the records in (b). (d) The inward (Na<sup>+</sup>) current seen in response to a 40 mV depolarising voltage-clamp step from a holding

potential of -70 mV (left) is blocked by TTX (25 μM, right). The voltage steps are shown above the current records. (e) Micrographs of magnetofected neurons (GFP<sup>+</sup>) used for whole-cell recording taken under normal transmitted light conditions (left) and a combination of low transmitted light and fluorescence excitation (right), also showing the recording and drug-application pipettes. Scale bars = 10 μm.





**Figure 4. Representative current clamp recordings from magnetofected (GFP<sup>+</sup>) and non-magnetofected (GFP<sup>-</sup>) neurons showing spike-like activity.** (a) Current steps (top) and corresponding voltage responses (middle) from a GFP<sup>+</sup> neuron. The arrows indicate spike-like voltage transients (blue arrows) and spontaneous depolarisations, probably reflecting synaptic

activity (black arrows). The bottom panel shows the response to the largest depolarising current injection on an expanded scale. A negative holding current was applied to hyperpolarise the neuron, the injection begins at 50 ms. (b). An equivalent recording from a GFP<sup>-</sup> neuron.

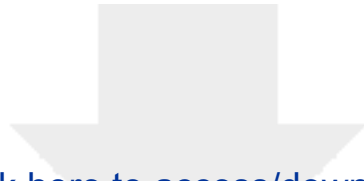
TABLES.

Table 1. Comparison of voltage-dependent Na<sup>+</sup> and K<sup>+</sup> currents in GFP<sup>+</sup> and GFP<sup>-</sup> (control) neurons in voltage-clamp.

	GFP positive (n = 8)	GFP negative (n = 6)
E <sub>m</sub> (mV)	-38 ± 1	-32 ± 3
I <sub>Na</sub> at -30 mV (pA)	-271 ± 88	-385 ± 144
I <sub>K</sub> at -30 mV (pA)	70 ± 20	33 ± 9
I <sub>K</sub> at 0 mV (pA)	806 ± 235	807 ± 257

**Table 1. Comparison of voltage-dependent Na<sup>+</sup> and K<sup>+</sup> currents in GFP<sup>+</sup> and GFP<sup>-</sup> neurons in voltage-clamp.** Current amplitudes were measured at the voltages indicated. For the measurements made at -30 mV, records were made using leak subtracted records. Membrane potential (E<sub>m</sub>) measurements are also shown, measured by linear interpolation of I-V curves at I = 0. There were no significant differences between the means for GFP<sup>+</sup> and GFP<sup>-</sup> neurons (Student's *t* test). Values are mean ± SEM, given to the nearest whole number.





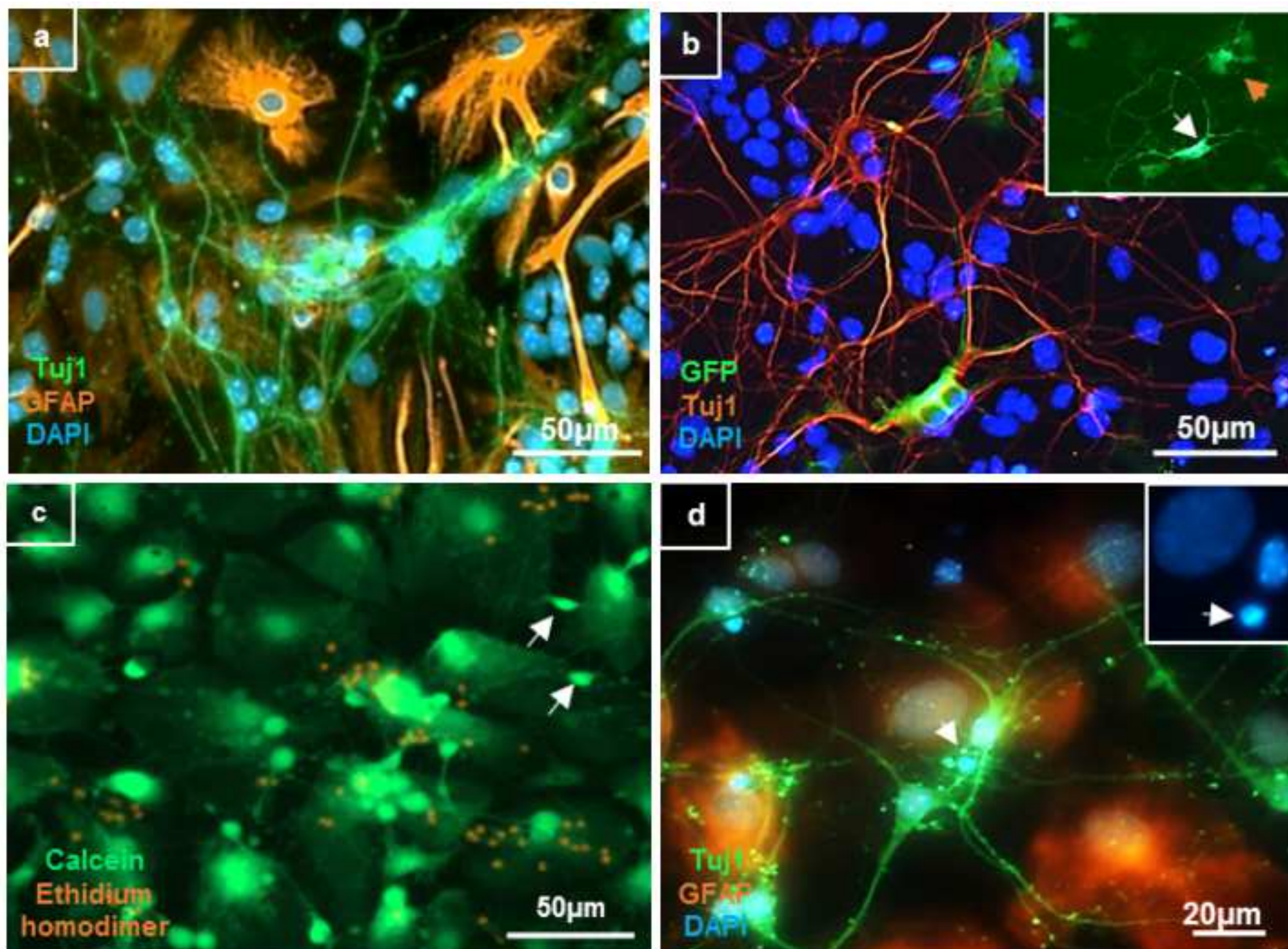
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Figure 1



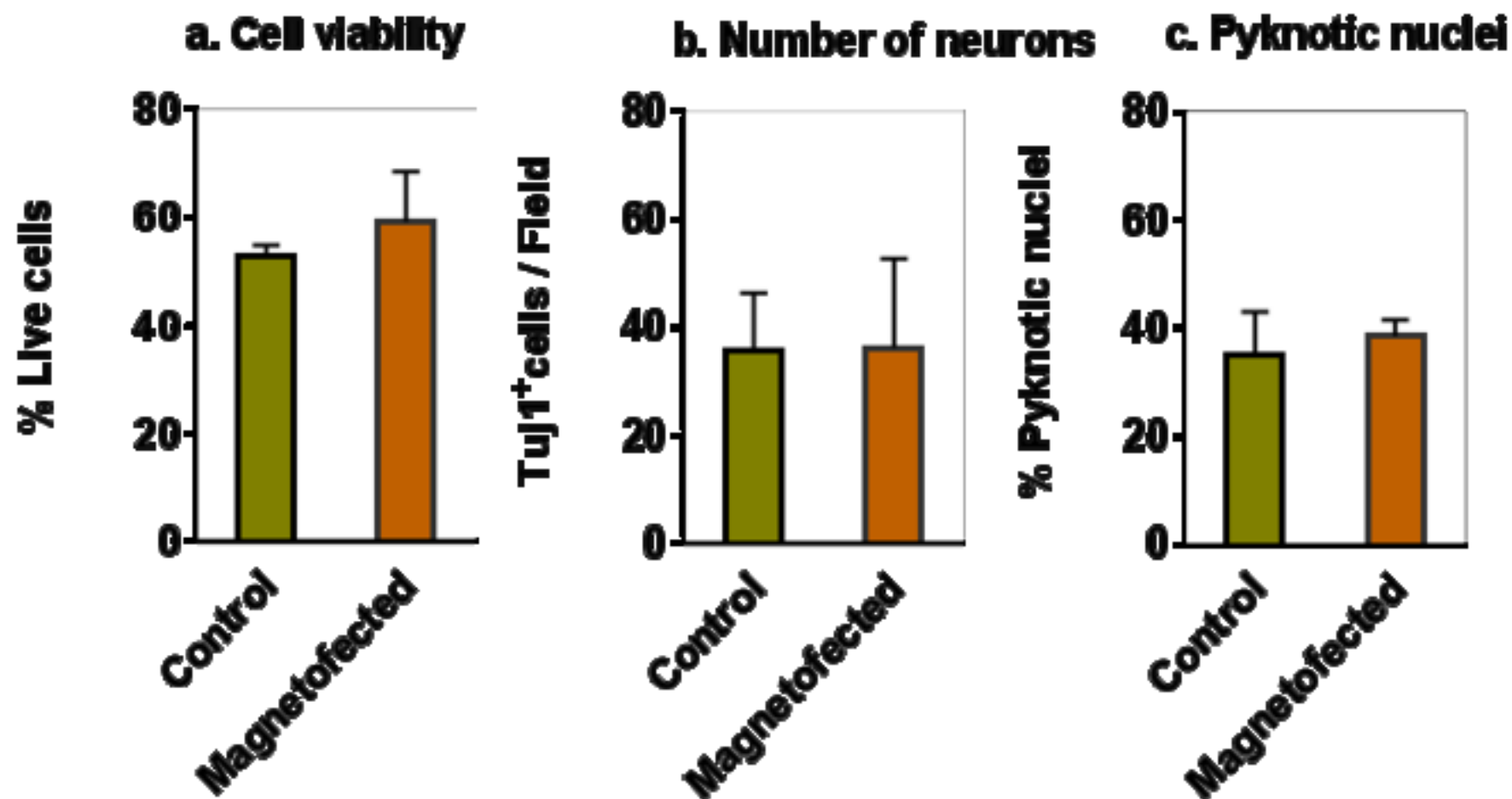


Figure 3

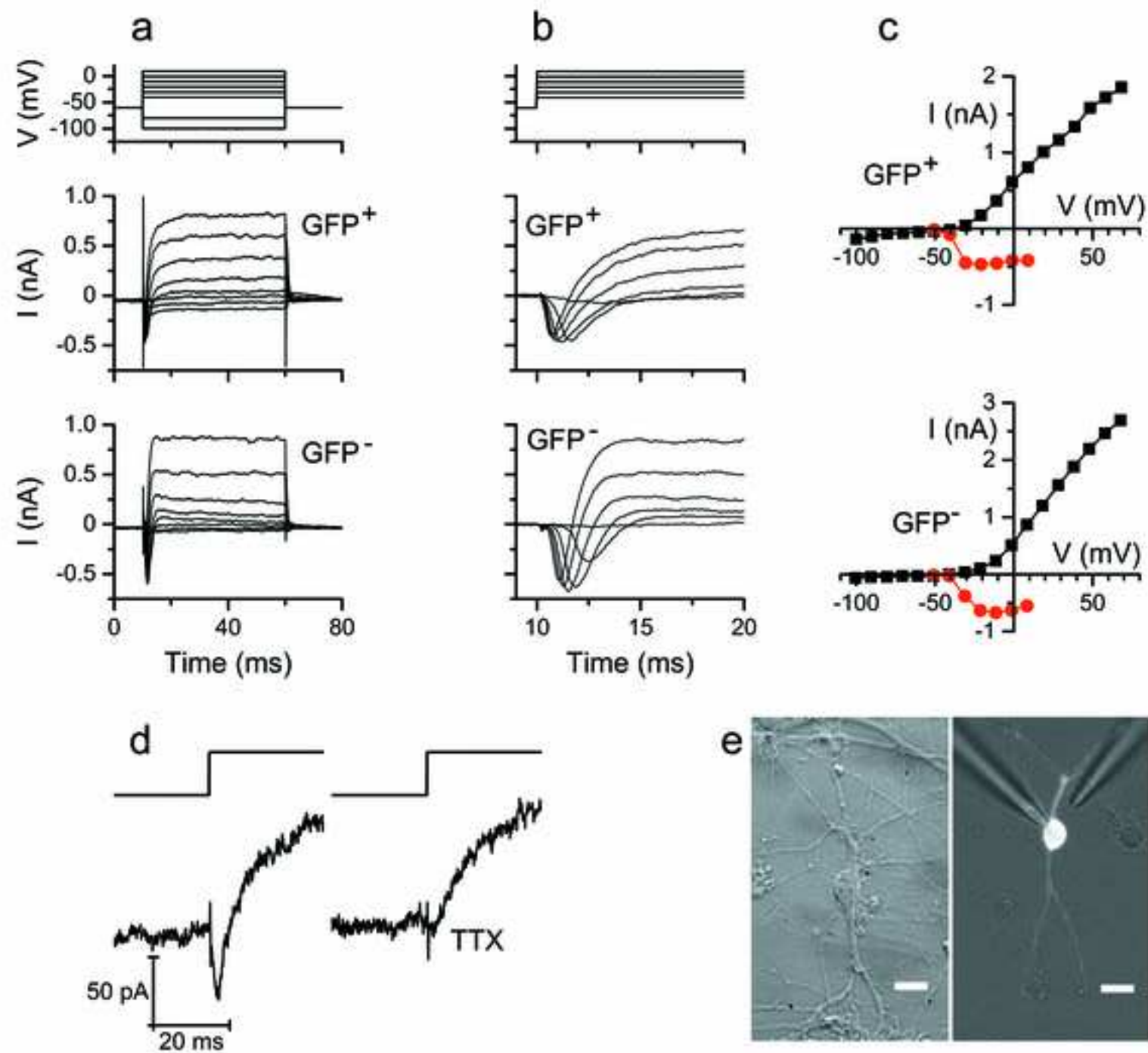




Figure 4

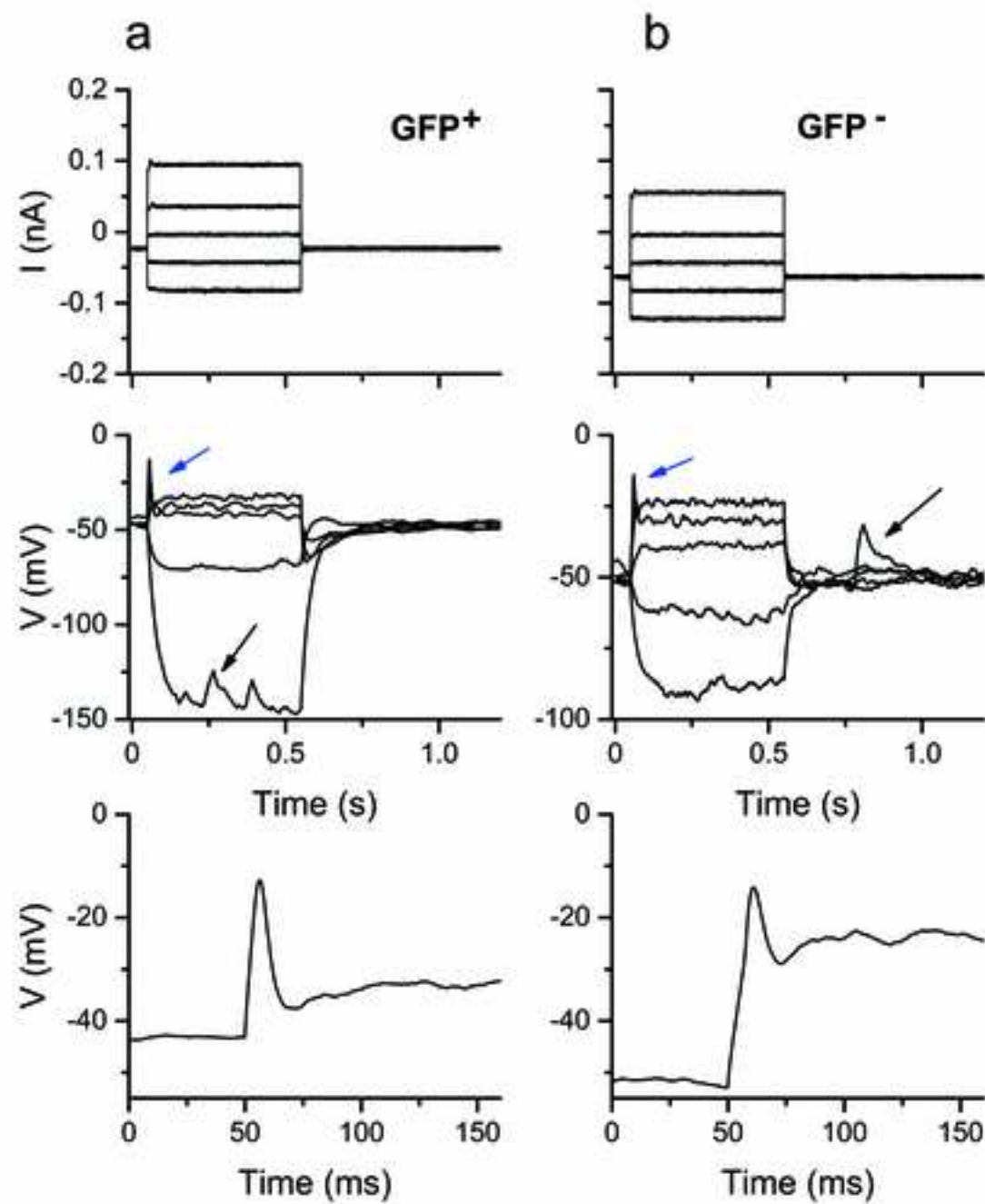
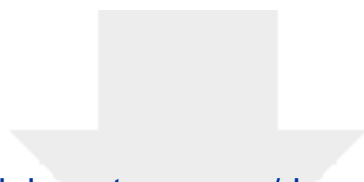


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